COMPARITIVE EFFICACY OF VESICULAR ARBUSCULAR MYCORRHIZA AND P-SOLUBILIZING BACTERIA IN SOME PLANTS

Research Protocol

For Registration for Ph.D. Programme

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2013

JECRC UNIVERSITY, JAIPUR

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INTRODUCTION

Plant nutrients are essential for the production of crops and healthy food for world's expanding population. Therefore, they are a vital component of sustainable agriculture[1].For optimum plant growth, nutrients must be available in sufficient and balanced quantities. Soil contain natural reserves of plant nutrients, but these reserves are largely in forms unavailable to plants, and only a minor portion is released each year through biological activity or chemical processes. Hence, fertilizers are designed to supplement the nutrients already present in the soil. The use of chemical fertilizer has its advantages and disadvantages in context of nutrient supply, crop growth and environmental quality [1].

In intensive cropping systems, supplementing soil nutrients by using chemical fertilizer is considered inevitable for obtaining optimum yield of crops. But it has been observed that continuous use of chemical fertilizers may affect soil health and may lead to a negative impact on soil productivity. Hence, for sustainable agriculture, all our efforts should be streamlined to protect and maintain soil health. In this context, now-a-days biofertilizers are gaining importance in agriculture[2].

In current scenario using chemical fertilizers in agricultural lands causes environmental problems including water supply contamination, shortage in quality of agricultural products and decrease in the amount of soil fertility[3]. Bio fertilizers not only apply to organic material resulted of animal residues, plant residues and so on. But they also include products resulted from microorganism's activities that act in relation to nitrogen stabilization or phosphorus and other nutrient elements preparation that act in soil[1]. One of the available ways to reach permanent agriculture is, using some microorganisms that have important role in supplying food requirement like mycorrhizal fungi and phosphate solubilizing bacteria[3]. Vesicular-arbuscular mycorrhizal fungi (VAM) has symbiotic correlation with many plants and it helps in decreasing absorption of heavy metals, increasing plant's resistance to disease elements of root, increasing phyto hormonal products, increasing the growth of hairy roots, aggravate the yield of nitrogen stability and with aggravating the amount of photosynthesis increases host plant's growth. Various factors which influence this ability of VAM are; amount of external mycelium distributed in soil and penetration into roots, environmental factors like light intensity, soil acidity and soil ventilation. The VAM fungi also helps in absorption of food elements like phosphorus and nitrogen and also absorbing water in tension condition, producing plant

hormones, balancing the effect of environmental tensions, increasing resistance to disease elements in plant, decreasing root's damages and repairing quality and quantity properties of agricultural products. Phosphorus is the most important element that is absorbed by mycorrhiza actively. Results of some researches show that the speed of phosphorus flow in mycorrhizal plant is 3 to 6 times more than non-mycorrhizal plants. In addition to phosphorus, nitrogen is also an element that increases its absorbance by mycorrhizal plants [3].

VAM extend the plant root system and the whole mycorrhiza (fungus plus plant) can exploit the soil nutrients much more effectively than the plant alone. Some plant nutrients like Phosphorus (P) and Zinc (Zn) move very slowly in the soil solution and therefore, the plant absorbs these nutrients from soil near the roots. A nutrient depletion zone may occur near the root and slow down plant nutrient uptake. These fungi grow out into the soil, sometimes several centimetres from the root and pick up nutrients at a distance where they are still readily available. The fungal strands (hyphae) then transport these nutrients quickly back to the plant – a kind of rapid transit system - overcoming the slow movement in the soil. Tolerance to drought can be increased as the rapid transit system overcomes slow movement of nutrients in dry soil [4].

VAM hyphae grow out of the roots and bind soil particles together, like a ‘sticky string bag’. This improves soil stability and can help to prevent erosion. The benefits do not come absolutely free, because the fungus needs sugars provided by the plant. Under most conditions, the plant produces sugars to spare, so the ‘cost’ of supporting the fungi is well invested. This results in enhanced nutrient uptake and more effective use of fertilizers. VAM are adapted to survive as resting stages in most soil types and conditions all around the world, including hot, dry, wet and frozen soils. They are present in soils of all textures, from sandy soils to those with high-clay content and are also present at a wide range of soil pH[4].

The supply of available nitrogen and phosphorous is commonly a limiting factor in crop production. The biological return of these nutrients to soil requires the presence of large population of suitable microorganism as well as environmental conditions conductive for the growth and activities of the organisms. The continuous drain of nitrogen and phosphorous in soil and the necessity for immense crop yield have led to an ever increasing emphasis upon means to utilize cheaply available and less

costly resources like native rock phosphate, atmospheric nitrogen etc. to their maximum potentiality[5].

Phosphorus application not only increases the plant biomass but also improves its protein contents. The deficiency of phosphorus in legumes depresses the activity of nitrogen fixing bacteria which reduces the availability of nitrogen in root zone. The combined application of Phosphorus (P) and Potassium (K) increased the weight of individual nodule in clover plant upto four times over control [6].

Soil microorganisms have great potential in providing soil phosphates for plant growth. Phosphorus supply through biological means as a viable substitute are phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF) and actinomycetes, which have been reported to be active in conversion of insoluble phosphate to soluble primary and secondary orthophosphate ions. Phosphorus biofertilizers can help to increase the accessibility of accumulated phosphates for plant growth by solubilization. Moreover, microorganisms involved in P solubilization as well as better scavenging of soluble Phosphorus can enhance plant growth through biological nitrogen fixation (BNF), enhancing the availability of other essential elements by production of plant growth promoting substances . Apart from playing an important role in biogeochemical cycles these soil bacteria have been used for decades in crop production. Due to their colonization in the rhizosphere and active role in promoting plant growth these bacteria are also termed as plant growth promoting rhizobacteria (PGPR). Application of phosphorus along with PSB improves P uptake by plants and yield, representing that PSB solubilize phosphates and activate phosphorus in crop plants. In this respect, biofertilization technology has substantially minimized the production costs and evades the environmental hazards at the same time [7].

The total cultivated area in Rajasthan is 17.4 million hectare, constituting almost half of the total area of the state. Over 70% area is rainfed with average precipitation of 575mm.The soil is coarse and poor in fertility and the cropping intensity is 125%.Important crops of the state are pearl millet, maize, chickpea, mustard, cluster bean and spices[8].

In the arid region, the annual rainfall is between 500-560 mm and fluctuation in rainfall is of the order of 49 per cent[8]. Therefore, in areas where rainfall is below 500-560 mm, drought resistant types of crops like jowar, bajra etc. are grown during the monsoon season. Production of crop is extremely precarious. This will require

introduction of improved methods of grass land management and readjustment of cultivation of crops in a more stable form of land use.

The present study is undertaken to trace out the best combination of bio-inoculum of VAM and PSB for enhancing the crop productivity and quality suitable to agro-climatic conditions of Jaipur (Rajasthan).

PART LITERATURE REVIEW

Efficiency of Phosphorus fertilizer throughout the world is around 10-25% and concentration of bioavailable P in soil is very low reaching the level of 1.0 mg Kg^{-1} soil[9].It has been reported that a great proportion of phosphorus in chemical fertilizers becomes unavailable to plants after its application in the soil.A significant reduction in the use of phosphate fertilizer could be achieved if solubilization of soil-insoluble phosphorus is made available to crop plants [10]. Phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield [11]. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids, which by their hydroxyl and carboxyl groups chelate the cations bound to phosphate and converts it into soluble form [12]. However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture [11]. PSB render more phosphates in soluble form than required for their growth and metabolism by secreting organic acids and enzymes (*e. g.* phosphatases), the surplus is received bythe plants [11]. The interest in PSB has increased due to the prospective use of efficient strains as bio-inoculant (biofertilizers) components in organic agriculture, which is emerging as an alternative to chemical inputs in intensive agriculture [13]. Due to their colonization in the rhizosphere and active role in promoting plant growth these bacteria are also termed as plant growth promoting rhizobacteria (PGPR) [7]. Phosphorus causes early ripening in plants, decreases grain moisture and improves crop quality. Feeding plants with phosphorus creates deeper and more abundant roots.It is a major building block of DND molecules [14].

Biological fertilizers (Phosphate solubilizing bacteria) are considered as the most effective plant assistants to supply phosphorus at a favourable level. These fertilizers are produced by selection of beneficial soil microorganisms having highest efficiency

to enhance plant growth by providing nutrients in absorbable form[15]. In 1973 the beneficial effect of vesicular arbuscular mycorrhizae (VAM) fungi, on plant growth was first reported[16]. Vesicular arbuscular mycorrhizal fungi are important soil organism which belongs to phylum Glomeromycota and form symbiotic associations with majority of terrestrial plant species [17]. Mycorrhizal fungi are benefited with carbon substrate from plants and in turn the plants are provided with nutrients especially phosphorous compounds from soil solution through the hyphal network of the fungi apart from increased absorptive surface area of the roots [18]. The community of vesicular arbuscular mycorrhizal fungi determines the plant community structure of an area due to the response of individual plant species towards single or multiple species of arbuscular mycorrhizal fungi[17].

Application of phosphorus along with P-Solubilizing Bacteria improves P uptake by plants and yield represents that PSB solubilize phosphates and activate phosphorus in crop plants [19]. In this respect, biofertilization technology has substantially minimized the production costs and evades the environmental hazards at the same time [7]. P-solubilizing *Rhizobium leguminosarum* has been shown to increase the growth of maize and lettuce [20]. According to an experimental work 10 out of 37 experiments PSB inoculations resulted in 10-15% increase in crop yields of spinach and French bean [21]. Simultaneous application of *Rhizobium* with PSM or arbuscular mycorrhizae (AM) fungi has been shown to stimulate plant growth more than with their sole inoculation in certain situations when the soil is Phosphorus deficient[22]. Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock [19].The PSB and VAM fungi together could reduce P fertilizer application by 50% without any significant reduction of crop yield [23].

Although microbial inoculants are in use for improving soil fertility during the last century, but a meagre work has been reported on Phosphorus- Solubilization as compared to nitrogen fixation. To our knowledge, no attempts have been made so far to study the cumulative and individual effect of PSB and VAM fungi as potential future inoculants or Biofertilizers for plant production in Rajasthan (Jaipur). The present study is designed to study and find out the potential isolates of VAM and PSB as future biofertilizers on the basis of Phosphorous solubilizing capacity and Nitrogen fixing capacity and its relationship with quality of soil of the study region.

STATEMENT OF THE PROBLEM

“Comparative Efficacy of Vesicular Arbuscular Mycorrhiza (VAM) and P-Solubilizing Bacteria (PSB) in some Plants”

OBJECTIVES OF THE STUDY

- To collect soil samples from the rhizosphere of plants grown in farmlands of Jaipur region.
- To isolate dominant VAM (Vesicular-Arbuscular Mycorrhiza) species and PSB (Phosphorus Solubilizing Bacteria) species in soil sample.
- To identify dominant VAM (Vesicular-Arbuscular Mycorrhiza) species and PSB (Phosphorus Solubilizing Bacteria) species in soil sample.
- To inoculate the selected VAM and PSB species on the host plant.
- To study the growth parameters on host plant when VAM and PSB species are applied individually and in combination.

SCOPE OF THE STUDY

- The scope of the study is isolation, identification and characterization of VAM and PSB species from the soil sample and inoculation of selected VAM and PSB species on host plant.
- The present study will highlight the best combination of bioinoculum suitable to the agro-climatic conditions of Jaipur (Rajasthan).

SIGNIFICANCE

- Phosphorus solubilizing bacteria and Vesicular-Arbuscular Mycorrhiza are very effective for increasing the available P in soil aswell as the growth and yield of plant. So, exploitation of phosphate solubilizing bacteria as bio-fertilizer along with VAM has enormous potential for making use of unavailable fixed P in the soil and use natural reserves of phosphate rock.
- The study will establish the potential of efficient indigenous phosphate solubilizing bacterial isolate and VAM isolate as a reliable alternative in low capital agriculture.
- The study will help other researchers or farmers interested in production of the biofertilizers to be effective over wide range of crops.

LIMITATIONS

- The study will be restricted only to selected Vesicular Arbuscular Mycorrhiza species and selected Phosphate Solubilizing Bacteria species.

METHODOLOGY

Following procedure will be followed for the experimentation:-

Collection of soil samples: As it is known fact that VAM and PSB are associated with plants, the soil samples are collected from the farmlands of Jaipur region. Each farmland will be divided into four different zones. Soil samples will be collected randomly from each zone at a depth of 0-15 cm after scrapping the top 1cm layer of soil. The samples collected from different places in each sites are pooled and homogenized. The collected soil and root samples will be stored in sealed plastic bags and transported to the laboratory in an insulated container. Before processing, all the samples are sieved (<2mm mesh size) to remove stones, coarse roots and other litter, and fine roots will be collected from each sample.

Isolation of VAM (vesicular arbuscular mycorrhiza) species and PSB (Phosphate solubilizing bacteria species): From the above collected soil samples the spores of VAM fungi will be isolated by the wet sieving and decanting technique[24]and the Phosphate solubilizing bacteria will be isolated by serial dilution and spread plate method using Pikovskaya's agar medium [25].

Identification of (vesicular arbuscular mycorrhiza) species and PSB (Phosphate solubilizing bacteria) species: VAM fungal spores will be examined and counted under stereomicroscope and identifications will be made by observing diagnostic characteristics such as spore wall, colour, size and type of hyphal attachment [26]. The synoptic keys of Trappe(1982) will be used to study the genera and species of Zygomycetous mycorrhizal fungi. The PVK medium will be used for isolation of PSB due to the presence of Calcium Triphosphate which is known for halozone formation [27]. Morphological and microscopic characteristics of isolates viz. shape, size, elevation, surface form, margins and surface texture, color, gram staining and Biochemical tests will be observed for their characterization[26].

Analysis of soil sample before inoculation of VAM and PSB: As it is known fact that VAM and PSB association with plant is related to the physiochemical and biological properties of the land therefore analysis of soil samples will be done on various parameters like pH (digital pH meter),Organic Carbon by Chromic Acid Method[28], Nitrogen by Kjeldahl Method[29], Phosphorous Estimation by Olsen's Method [30], estimation of Calcium carbonate by Loeppert method [31].

Soil Analysis after inoculation of VAM and PSB species:

The next step of experiment will be carried out in mud pot .The plants will be selected during the study which are grown in the farmlands of Jaipur during summer season. Here, the soil samples (without VAM Fungi and PSB) will be transferred to the mud pots. Before transferring, the soil will be steam sterilized for 2 hours for two consecutive days. The sterile soil in pots will be inoculated with 5-10% of starter inoculums as a layer of two inches below the soil level and surface sterilized seeds of host plants will be sowed. The seedlings will be periodically watered and the relative efficiency of different VAM species and PSB species will be studied on various growth parameters (viz. Biochemical) along with control conditions.

Biochemical Studies: The quantitative and qualitative estimation of various biochemicals will be done on same plant as compared with control. The various test are as follows: -

-Estimation of Starch [32]

-Estimation of total Phenol [33]

-Estimation of reducing sugar [34]

-Estimation of non-reducing sugar [35]

-Extraction and estimation of Chlorophyll [36]

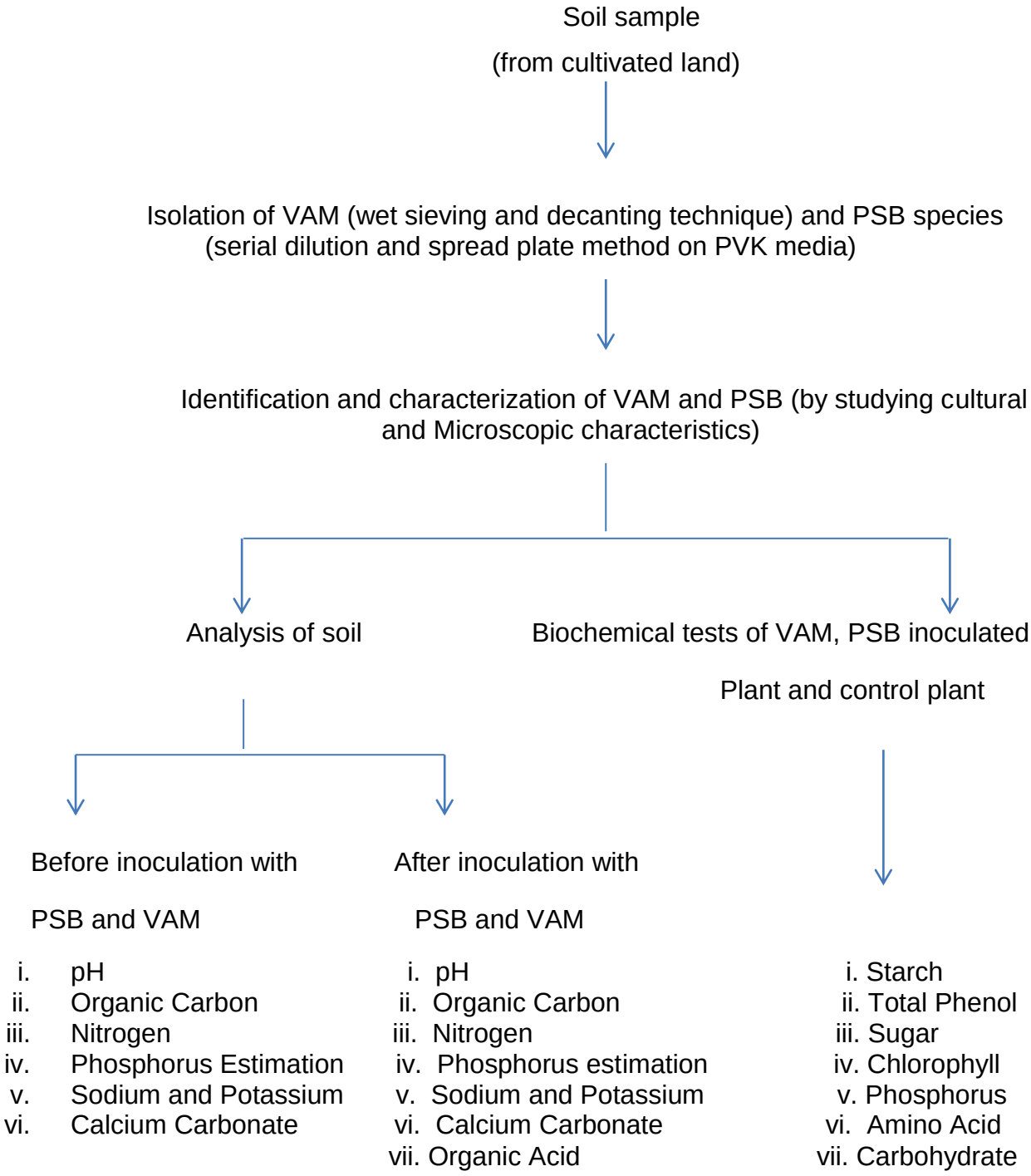
-Estimation of phosphorous [30]

-Extraction and estimation of free amino acids [37]

-Estimation of Ascorbic acid [37]

-Identification of Organic acid production (HPLC technique).

OUTLAY


Soil sample
(from cultivated land)
Isolation of VAM (wet sieving and decanting technique) and PSB species (serial dilution and spread plate method on PVK media)
Identification and characterization of VAM and PSB (by studying cultural and Microscopic characteristics)
Analysis of soil
Biochemical tests of VAM, PSB inoculated Plant and control plant
Before inoculation with PSB and VAM
i. pH
ii. Organic Carbon
iii. Nitrogen
iv. Phosphorus Estimation
v. Sodium and Potassium
vi. Calcium Carbonate
After inoculation with PSB and VAM
i. pH
ii. Organic Carbon
iii. Nitrogen
iv. Phosphorus estimation
v. Sodium and Potassium
vi. Calcium Carbonate
vii. Organic Acid
i. Starch
ii. Total Phenol
iii. Sugar
iv. Chlorophyll
v. Phosphorus
vi. Amino Acid
vii. Carbohydrate

YEAR WISE PLAN OF WORK

1-24 months	Review of Literature throughout the study
1-3 months	Sample Collection
3-6 months	Isolation and Identification of VAM and PSB species
6-12 months	Ecophysiological and Biochemical Characterization of VAM and PSB inoculated plant and control plant.
12-20 months	Analysis and Interpretation of results
20-24 months	Thesis Preparation

TOTAL TIME (EXPECTED): 24 Months

Work schedule (Months wise)

Months→ Activity↓	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Review of Literature																								
Sample collection																								
Isolation and Identification of VAM and PSB species																								
Ecophysiological and Biochemical Characterization of VAM and PSB inoculated plants																								
Analysis and Interpretation of Data																								
Preparation and Submission of Report																								

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